Version With Markings to Show Changes Made

73. (Once amended) An [insect control] agent comprising a baculovirus, wherein the baculovirus directs transcription of at least one ribonucleic acid (RNA) that, when present within a [an insect] cell, forms a double-stranded structure that inhibits expression of at least one [insect] gene.

75. (Once amended) An agent comprising a baculovirus vector, wherein the baculovirus vector directs transcription of at least one ribonucleic acid (RNA) that, when present within a cell, forms a double-stranded structure that inhibits expression of at least one gene, wherein the RNA comprises two separate complementary strands, and [The agent of claim 74,] wherein the baculovirus expression vector comprises a DNA segment flanked by two promoters, wherein the promoters are operably linked to the DNA segment, and wherein the promoters are oriented so as to direct transcription of both sense and antisense RNA transcripts from the flanked DNA segment.

78. (Once amended) An agent comprising a baculovirus, wherein the baculovirus directs transcription of at least one ribonucleic acid (RNA) that, when present within a cell, forms a double-stranded structure that inhibits expression of at least one gene, [The agent of claim 73] wherein the RNA comprises one strand that is self-complementary.

79. (Once amended) The agent of claim 73 or 74, wherein at least a portion of the ribonucleic acid sequence is substantially identical to at least a portion of the sequence of the at least one gene.

80. (Once amended) The agent of claim 73 or 74, wherein the cell forms part of a tissue of a target pest organism.

81. (Once amended) The agent of claim 73 or 74, wherein the baculovirus is selected from the group consisting of: the *Autographa californica* multiple polyhedrosis virus, the *Orgyia pseudotsugata* MNPV, the *Lymantria dispar* MNPV, the *Helicoverpa zea* NPV, and the *Bombyx mori* NPV.

82. (Once amended) The agent of claim 73 or 74, wherein the at least one gene to be inhibited is an essential gene in an [the] insect.

83. (Once amended) The agent of claim 73 or 74, wherein at least one gene to be inhibited is a gene involved in development in a pest organism.

84. (Once amended) The agent of claim 73 or 74, wherein at least one gene to be inhibited is involved in neurotransmission in a pest organism.

85. (Once amended) The agent of claim 73 or 74, wherein at least one gene to be inhibited is expressed in the insect alimentary canal or Malpighian tubules.

86. (Once amended) The agent of claim 73 or 74, wherein the at least one gene to be inhibited is naturally found in an insect selected from the order *Lepidoptera*.

87. (Once amended) The agent of claim 73 or 74, wherein the at least one gene to be inhibited is naturally found in an insect selected from the list consisting of: the cotton bollworm (*Helicoverpa zea*), the cabbage looper (*Trichoplusia ni*), the alfalfa looper (*Autographa californica*), the tobacco hornworm (*Manduca sexta*), the tobacco budworm (*Heliothis virescens*), the fall armyworm (*Spodoptera frugiperda*), the European corn borer (*Ostrinia nubilalis*), the eastern spruce budworm (*Choristoneura fumiferana*), the western spruce budworm (*C. occidentalis*), and the gypsy moth (*Lymantria dispar*).

90. (Once amended) An insecticidal composition comprising the agent of claim 73 or 74,

wherein the gene is an insect gene, and an agriculturally suitable carrier.

92. (Once amended) A recombinant baculovirus comprising:

a first promoter;

a second promoter;

a DNA segment whose sequence comprises at least one sequence at least 80% identical to a portion of the sequence of at least one insect gene, wherein the at least one insect gene is selected from the list consisting of: genes that are essential in a pest organism, genes involved in neurotransmission in a pest organism, genes involved in development in a pest organism, and genes expressed in the insect alimentary canal or Malpighian tubules, and wherein the portion of the sequence of the at least one insect gene is at least 50 nucleotides in length;

a first enhancer operably linked to the first promoter,

a second enhancer operably linked to the second promoter,

a first transcriptional terminator, wherein the first transcriptional terminator is positioned so as to terminate transcription directed by the first promoter;

a second transcriptional terminator, wherein the second transcriptional terminator is positioned so as to terminate transcription directed by the second promoter, wherein the two promoters are operably linked to the DNA segment, and wherein the promoters are oriented so as to direct transcription of both sense and antisense RNA transcripts from the DNA segment.

123 [192]. (Once amended) The pest control agent of any of claims 111, 112, and 113, wherein the baculovirus is selected from the group consisting of: the *Autographa californica* multiple polyhedrosis virus, the *Orgyia pseudotsugata* MNPV, the *Lymantria dispar* MNPV, the *Helicoverpa zea* NPV, and the *Bombyx mori* NPV.

131. (Once amended) A method of controlling insects, the method comprising the step of: contacting a cell in an insect with a first ribonucleic acid (RNA) whose sequence corresponds to at least a portion of at least one gene endogenous to the insect, wherein the first ribonucleic acid hybridizes either with itself or with a second ribonucleic acid with which the cell is also contacted, thereby forming a double-stranded structure within the cell that inhibits expression of

at least one gene expressed in the cell, wherein the step of contacting comprises contacting the insect with a baculovirus, and [The method of claim 130,] wherein the first RNA is expressed within 6 hours after the insect is contacted with the baculovirus.

132. (Once amended) A method of controlling insects, the method comprising the step of: contacting a cell in an insect with a first ribonucleic acid (RNA) whose sequence corresponds to at least a portion of at least one gene endogenous to the insect, wherein the first ribonucleic acid hybridizes either with itself or with a second ribonucleic acid with which the cell is also contacted, thereby forming a double-stranded structure within the cell that inhibits expression of at least one gene expressed in the cell, wherein the step of contacting comprises contacting the insect with a baculovirus, and [The method of claim 130,] wherein the RNA is expressed substantially in the absence of viral replication.

133. (Once amended) A method of controlling insects, the method comprising the step of: contacting a cell in an insect with a first ribonucleic acid (RNA) whose sequence corresponds to at least a portion of at least one gene endogenous to the insect, wherein the first ribonucleic acid hybridizes either with itself or with a second ribonucleic acid with which the cell is also contacted, thereby forming a double-stranded structure within the cell that inhibits expression of at least one gene expressed in the cell, wherein the step of contacting comprises contacting the insect with a baculovirus, and [The method of claim 130,] wherein the baculovirus does not establish a productive infection.

134. (Once amended) The method of claim 131 [129], wherein the insect is a *Lepidopteran*.

138. (Once amended) The method of claim 131 [any of claims 126, 127, and 128], wherein the gene is selected from the group consisting of: genes that are essential in a pest organism, genes involved in neurotransmission in a pest organism, genes involved in development in a pest organism, and genes expressed in the insect alimentary canal or Malpighian tubules.

139. (Once amended) The method of claim 131 [130], wherein the baculovirus is selected from the group consisting of: the *Autographa californica* multiple polyhedrosis virus, the *Orgyia pseudotsugata* MNPV, the *Lymantria dispar* MNPV, the *Helicoverpa zea* NPV, and the *Bombyx mori* NPV.

140. (Once amended) The method of claim 131 [130], wherein the step of contacting comprises applying the baculovirus to organisms on which the insect feeds.

141. (Once amended) The method of claim 131 [any of claims 126, 127, or 128], whereby one or more biological or physiological functions of the insect is inhibited.

Remarks

Claim 73 has been amended to indicate that the baculovirus of the invention need not be considered an insect control agent, and the gene whose transcription is directed by the baculovirus need not inhibit an insect gene but rather inhibits a gene in any cell type in which the baculovirus is introduced, provided that the key requirements for inhibition by dsRNA, e.g., formation of a double-stranded structure, appropriate degree of sequence identity with the target gene, etc., are satisfied (see, e.g., paragraph bridging pages 12 and 13, and p. 27, lines 9-13)

As described in the specification, the baculovirus of the invention directs transcription either of two complementary RNA strands or of a single, self-complementary RNA strand, wherein the template(s) for such strand(s) are contained within a suitable plasmid, e.g., the plasmids of the invention (See paragraph bridging pages 19 and 20). These plasmids are described on p. 15 (lines 13-26) and at the paragraph bridging pages 35 – 36 of the specification. It will be appreciated that any DNA segment, not necessarily DNA segments corresponding to an insect gene, may be introduced into the "DNA region into which a DNA segment can be inserted", which is present in the inventive plasmids. The inventive plasmids containing any DNA segment may be introduced into the baculovirus, wherein transcription will occur.

The invention encompasses introduction of inventive baculoviruses into non-insect cells. (See p. 31, lines 9-10, referring to an article by Carbonell, et al., describing baculovirus-mediated gene expression in dipteran and mammalian cells). Furthermore, the ability of baculoviruses to direct gene expression in a wide range of cell types, including cell types in which they are unable to establish a productive infection, was well known in the art at the time the application was filed. See, for example, Boyce, F. and Buchner, N., "Baculovirus-mediated gene transfer into mammalian cells" *Proc Natl Acad Sci U S A* Mar 19;93(6):2348-52, 1996; Sandig,V, et al., "Gene transfer into hepatocytes and human liver tissue by baculovirus vectors" *Human Gene Ther* 20,1937-45, 1996.

In addition, the application encompasses the fact that dsRNA operates to inhibit gene expression in a wide variety of cell types, as recognized by Fire, the inventor of dsRNA inhibition. Fire's work is referenced on p. 13, lines 2-3 and p. 14, line 2. Furthermore, Fire's PCT application (WO99/32619), published 7/1/99, cited by the Examiner and by Applicant, clearly applies dsRNA inhibition to any cell type, as do the claims in Fire's issued U.S. patent (U.S.S.N. 6,506,559). Thus Applicants submit that claim 73 as amended is fully supported by

the specification as filed. The Examiner's rejection of original claim 73 is discussed below.

Claims 79-87 and 90 have been amended to indicate that these claims may depend from claim 74 in addition to claim 73. This dependency is clearly supported by the specification, as it simply indicates that the "at least one ribonucleic acid (RNA)" whose transcription is directed by the baculovirus may comprise two separate, complementary strands, as described throughout the specification and summarized on p. 35, lines 14-16. Claim 90 has been further amended to indicate that the gene is an insect gene.

Claim 123 has been amended to correct the claim number, which was incorrectly listed as 192 in the original claim.

New claim 142 is essentially identical to original claim 73.

New claims 143 – 151 are essentially identical to original claims 79-90, except that they depend on claims 75 or 78 rather than claim 73 as originally and some claims have been consolidated. These claims were added because claims 75 and 78 have been rewritten as independent claims to overcome the Examiner's objection, rather than being dependent on claim 73 as originally.

Rejections under 35 U.S.C. § 102

The Examiner has rejected claims 73-74, 79-87, and 90 as being anticipated by Adams et al. The Examiner states that Adams recites pest control agents comprising an expression vector containing sequences encoding antisense (and optionally sense) RNAs targeted against (and complementary to) an insect gene. However, Adams does not teach the limitations of claim 73, namely that the RNA *forms a double-stranded structure that inhibits expression of at least one gene*. Adams nowhere mentions the possibility of formation of a double-stranded structure, and it is reading far too much into Adams to argue that his invention encompasses formation of a double-stranded structure in a cell.

Adams describes traditional antisense approaches to inhibiting gene expression while Applicants employ the newly discovered technique of double-stranded RNA inhibition. Adams describes a variety of genes encoding hormones that are involved in insect development, particularly ecdysis (shedding the old cuticle). These genes encode ETH, ETH-PRP2, ETH-PRP3, etc. Adams points out that either inappropriate expression of these genes *or* inhibition of these genes can disrupt or interfere with insect development. For example, Adams states that "ETH has been found...to disrupt development of all insect life stages" (col. 9, lines 4-5), and

Example 18 states that, "Expression of insect hormones such as ETH at the inappropriate time will result in more rapid cessation of feeding and development" (col. 49, lines 43-45). Thus Adams contemplates interfering with insect development by overexpressing ETH. Conversely, Example 18 also describes separate antisense constructs, targeted against the same genes, that would "deliver anti-hormone sequences to pests and prevent successful molting of the insect." (col. 49, lines 63-65). Example 18 clearly distinguishes between constructs comprising sense sequences for overexpression and constructs comprising antisense sequences for inhibiting expression.

In col. 9, lines 59-63, Adams states that, "a sense, an antisense, or combination of sense and antisense sequences for one or a combination of the ETH, ETH-PRP1, ETH-PRP2...molecules will be engineered into a virus". This highly general language cannot be said to teach a virus that contains both sense and antisense sequences for a single gene, as required by Applicants' invention. Indeed the following column (col. 10, lines 10-18) makes it clear that Adams envisions the sense and antisense approaches as separate and distinct. Adams nowhere suggests that sense and antisense sequences should form a double-stranded structure. He simply does not envision the presence of antisense and sense sequences for a single gene within the same virus. In summary, Adams contemplates controlling insect either by overexpressing certain genes *or* by inhibiting these genes using antisense RNA, while Applicants' contemplate inhibition only, by way of double-stranded RNA.

The baculoviruses of Adams may contain sense and/or antisense sequences of one or more of the genes that he describes. However, Adams does not describe a baculovirus having sense and antisense sequences corresponding to *the same gene*, which is an essential feature of Applicants' invention. Instead, Adams envisions at most baculovirus that contains sense sequence(s) corresponding to certain of the genes he identifies, for purposes of causing overexpression of these genes, and antisense sequence(s) corresponding to *different genes* from among those he mentions, for purposes of causing inhibition of those particular genes. In other words, according to the teachings of Adams, given their broadest possible interpretation, the same baculovirus may cause overexpression and inhibition of *different* genes. Overexpression is may be caused by the presence of sense sequences while inhibition *of other genes* may be caused by presence of antisense sequences. It would be completely illogical for the baculoviruses of Adams to include both sense and antisense sequences corresponding to the same gene, as required in Applicants' baculoviruses. According to the teachings of Adams, the sense sequence

would cause overexpression of the gene while the antisense sequence would cause inhibition, leading to a canceling out effect. Thus the invention of Adams only contemplates baculoviruses in which either (1) only sense sequences are present; (2) only antisense sequences are present; or (3) sense and antisense sequences *corresponding to different insect genes* are present.

The Examiner has also stated that Applicants and Adams et al. recite methods of controlling insect pests comprising inhibiting the expression of an essential gene in said pest by introducing into cells of said pest a vector which expresses antisense sequences targeted against said essential gene. However, as described above, unlike Adams, Applicants' invention does not rely on traditional antisense approaches to inhibit genes. The baculoviruses of Applicants' invention do not simply direct transcription of an antisense RNA targeted against the gene to be inhibited. Instead, in accordance with claim 73, the antisense RNA is always accompanied by a complementary sequence, *so that a double-stranded RNA is formed.* Thus Applicants do not teach or claim inhibition of genes using antisense sequences. Applicants' baculoviruses always direct transcription of both complementary strands, not merely an antisense strand corresponding to the gene to be inhibited.

In summary, Applicants respectfully submit that Adams does not anticipate claim 73 either as originally present in the application or as amended. Since claims 74, 79-87, and 90 depend either directly or indirectly on claim 73, Applicants submit that these claims are also allowable.

The Examiner has rejected claims 126-129, 134, 138, and 141 as being anticipated by Fire et al. Claims 126-129 have been cancelled. Claims 134, 138 and 141 have been amended to depend from claim 131, and Applicant submits that the amended claim is not anticipated by Fire since claim 131 was not anticipated by Fire et al.

Rejections under 35 U.S.C. § 103

The Examiner has rejected claim 91 as being unpatentable over Adams et al., in view of Miller, et al. Claim 91 is dependent on claim 90, which is in turn dependent on claim 73 (and, as amended is now also dependent on claim 74). The Examiner asserted that Adams taught the invention of claim 73, and Miller contributes the teaching of agriculturally suitable carriers. As discussed above, Applicants submit that Adams does not teach the invention of claim 73 either as originally present in the application or as amended. Therefore, Applicants respectfully submit

that the combination of Adams and Miller does not render claim 91 obvious.

The Examiner has rejected claims 130, 135, and 139-140 as being unpatentable over Fire et al. in view of Adams et al.

Claims 130 and 135 have been cancelled.

Claim 139 has been amended to be dependent on claim 131 rather than on rejected claim 130. Claim 131 has been amended to overcome the Examiner's objection. Applicant submits that since claim 131 is not obvious in view of Fire and Adams, claims 138 and 139 as amended are also not obvious.

Rejections under 35 U.S.C. § 112

The Examiner has rejected claims 88-89, 94-95, 107-108, 120-121 and 136-137 as containing subject matter that is not adequately described in the specification. All of these claims have been cancelled.

Rejections under 35 U.S.C. § 101

The Examiner rejected claim 92 for double patenting as claiming the same invention as that of claim 1 of Applicants' prior U.S. Patent No. 6,326,193. Claim 92 has been amended to include the limitations of claim 93, and Applicants submit that it therefore does not claim the same invention as that in claim 1 of the '193 patent.

Objections

The Examiner objected to claims 80, 83, and 84 under 37 C.F.R. § 1.75(c) as being of improper dependent form for failing to limit the subject matter of a previous claim in that claim 73 recited an "insect control agent" whereas claims 80, 83, and 84 recited that the target cell or gene is from a "target pest organism" which is broader in scope. As discussed above, claim 73 has been amended to remove the phrase "insect control". Applicants submit, therefore, that claims 80, 83, and 84 are therefore no longer of improper dependent form.

The Examiner objected to claims 75-78, 93, 96-106, 109-110, and 130-133 as being dependent upon rejected base claims and indicated that these claims would be allowable if rewritten to include the limitations of the base claim and any intervening claims.

Claims 75 and 78 have accordingly been rewritten to include the limitations of claim 73

as amended. Claims 76 and 77 are dependent on claim 75 and thus also now include the limitations of claim 73.

Claim 93 has been cancelled.

Claims 96 and 97 are dependent on claim 92, which was rejected for double patenting and has now been amended to overcome the rejection, as described above. Applicants submit that claims 96 and 97 are therefore allowable.

The Examiner stated that 98-106 and 109-110 are dependent on a rejected base claim. However, claim 98 is an independent claim that was itself objected to as being dependent on a rejected base claim. Claims 99-106 and 109-110 are dependent on claim 98. Applicant requests clarification regarding the Examiner's position with respect to these claims.

Claim 130 has been cancelled.

Claims 131-133 have been rewritten to include the limitations of their respective base claims. Applicants submit that these claims are now allowable.

Respectfully submitted,

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